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Chemicals
NEWS 3 JAN 16 CA/CAplus Company Name Thesaurus enhanced and reloaded NEWS 4 JAN 16 IPC version 2007.01 thesaurus available on STN NEWS 5 JAN 16 WPIDS/WPINDEX/WPIX enhanced with IPC 8 reclassification
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YOU HAVE REQUESTED DATA FROM 9 ANSWERS - CONTINUE? Y/(N):y
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AN 2007:175345 CAPLUS <<LOGINID::20070611>>
DN 146:222528
NEWS 6 JAN 22 CA/CAplus updated with revised CAS roles
NEWS 7 JAN 22 CA/CAplus enhanced with patent applications from India
 NEWS 8 JAN 29 PHAR reloaded with new search and display fields
                                                                                                                                                              TI Expression vector and methods of producing erythropoietin and other
NEWS 9 JAN 29 CAS Registry Number crossover limit increased to 300,000 in multiple databases
                                                                                                                                                              recombinant proteins in mammalian cells for potential therapeutic use IN Singh, Arun K.; Goel, Ashish; Mendiratta, Sanjeev K.
NEWS 10 FEB 15 PATDPASPC enhanced with Drug Approval numbers
NEWS 11 FEB 15 RUSSIAPAT enhanced with pre-1994 records
NEWS 12 FEB 23 KOREAPAT enhanced with IPC 8 features and functionality
                                                                                                                                                              PA Cadila Healthcare Limited, India
SO PCT Int. Appl., 33pp.
CODEN: PIXXD2
NEWS 12 FEB 23 KOREAPT enhanced with IPC 8 features and functionality
NEWS 13 FEB 26 MEDLINE reloaded with enhancements
NEWS 14 FEB 26 EMBASE enhanced with Clinical Trial Number field
NEWS 15 FEB 26 TOXCENTER enhanced with reloaded MEDLINE
NEWS 16 FEB 26 IFICDB/IFIPAT/IFIUDB reloaded with enhancements
NEWS 17 FEB 26 CAS Registry Number crossover limit increased from 10,000
to 300,000 in multiple databases
NEWS 18 MAR 15 WPIDS/WPIX enhanced with new FRAGHITSTR display
                                                                                                                                                              DT Patent
                                                                                                                                                               LA English
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                                                                                                                                                                                                                                 APPLICATION NO.
                                                                                                                                                                   PATENT NO.
                                                                                                                                                                                                   KIND DATE
                                                                                                                                                                       WO 2007017903
format
NEWS 19 MAR 16 CASREACT coverage extended
NEWS 20 MAR 20 MARPAT now updated daily
NEWS 21 MAR 22 LWPI reloaded
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RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
IN 2005-MU720 A 20050620
The present invention provides an expression vector and methods for
NEWS 22 MAR 30 RDISCLOSURE reloaded with enhancements NEWS 23 APR 02 JICST-EPLUS removed from database clusters and STN
 NEWS 24 APR 30 GENBANK reloaded and enhanced with Genome Project ID
NEWS 25 APR 30 CHEMCATS enhanced with 1.2 million new records
NEWS 26 APR 30 CA/CAplus enhanced with 1870-1889 U.S. patent records NEWS 27 APR 30 INPADOC replaced by INPADOCDB on STN NEWS 28 MAY 01 New CAS web site launched NEWS 29 MAY 08 CA/CAplus Indian patent publication number format defined NEWS 30 MAY 14 RDISCLOSURE on STN Easy enhanced with new search and
                                                                                                                                                              PRAI IN 2005-MU720 A 20050620

AB The present invention provides an expression vector and methods for overproducing erythropoietin and other recombinant proteins such as TNFR-IgGFc fusion proteins, rituximab, trastuzumab, bevacuzumab, or other monoclonal antibodies in mammalian cells. The expression vector comprises a ***CMV**** promoter or functional variants, an intron, TPL regulatory
NEWS 31 MAY 21 BIOSIS reloaded and enhanced with archival data
                                                                                                                                                                   element or its functional variants, VA genes or functional variants, and a bovine growth hormone polyadenylation element or functional variants. Upon stable transfection of CHO-DHFR- cells, the expression vector
 NEWS 32 MAY 21 TOXCENTER enhanced with BIOSIS reload
NEWS 33 MAY 21 CA/CAplus enhanced with additional kind codes for German
                  patents
NEWS 34 MAY 22 CA/CAplus enhanced with IPC reclassification in Japanese
                                                                                                                                                                   provides 11,830 IU/mL in 168 h culture, which is equiv. to 18.2 to 27.3 .mu.g/106 cells/24 h. This expression vectors provides 80-100% higher
                  patents
                                                                                                                                                                    expression of recombinant erythropoletin than those previously reported in
NEWS EXPRESS NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c.
                                                                                                                                                                   ther literature.
CURRENT
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              MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 25 SEPTEMBER 2006.
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NEWS HOURS STN Operating Hours Plus Help Desk Availability
NEWS LOGIN Welcome Banner and News Items
NEWS IPC8 For general information regarding STN implementation of IPC 8
                                                                                                                                                              DN PREV200700197446
                                                                                                                                                               TI Derivation and characterization of cholesterol-independent non-GS NS0 cell
                                                                                                                                                              Derivation and characterization of cholesterol-independent non-GS Nov cellines for production of recombinant antibodies.

AU Hartman, Taymar E. [Reprint Author]; Sar, Nalin; Genereux, Kimberly; Barritt, Diana S.; He, Yimir, Burky, John E.; Wesson, Mark C.; Tso, J. Yun; Tsurushita, Naoya; Zhou, Weichang; Sauer, Paul W.

CS PDL BioPharma Inc, Proc Sci and Engn, 34801 Campus Dr, Fremont, CA
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 of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.
                                                                                                                                                              SO Biotechnology and Bioengineering, (FEB 1 2007) Vol. 96, No. 2, pp.
                                                                                                                                                                   294-306
                                                                                                                                                                   CODEN: BIBIAU. ISSN: 0006-3592.
DT Article
LA English
                                                                                                                                                              ED Entered STN: 21 Mar 2007
FILE 'HOME' ENTERED AT 16:39:08 ON 11 JUN 2007
                                                                                                                                                              Last Updated on STN: 21 Mar 2007

AB Presented is an antibody production platform based on the fed-batch culture of recombinant NSO-derived cell lines. NSO host cells, obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK, Part
=> FIL BIOSIS CAPLUS EMBASE
COST IN U.S. DOLLARS
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                                                                                                                                                                   No. 85110503), were first adapted to grow in a protein-free; cholesterol-free medium. The resulting host cell line was designated NS0-PFCF (protein-free, cholesterol-free). The five production cell lines
FULL ESTIMATED COST
FILE 'BIOSIS' ENTERED AT 16:42:11 ON 11 JUN 2007
                                                                                                                                                                   presented here were generated using a common protocol consisting of transfection by electroporation and subcloning. The NS0-PFCF host cell line was transfected using a single expression vector containing the
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FILE 'CAPLUS' ENTERED AT 16:42:11 ON 11 JUN 2007

Escherichia coli xanthine-guanine phosphoribosyl transferase gene (gpt), and the antibody heavy and light chain genes driven by the ***CMV**** promoter. The five cell lines were chosen after one to three rounds of iterative subdon ing, which resulted in a 19-64% increase in antibody productivity when four mother-daughter cell pairs were cultured in a fed-batch bioreactor process. The production cell lines were genetically characterized to determine antibody gene integrity, nucleotide sequences, copy number, and the number of insertion sites in the NS0 cell genome. Genetic characterization data indicate that each of the five production cell lines, has a single stably integrated copy of the antibody expression vector, and that the antibody genes are correctly expressed. Stability of antibody production was evaluated for three of the five cell lines by comparing the early stage seed bank with the Working Cell Bank (WCB). Antibody productivity was shown to be stable in two of three cell lines evaluated; while one of the cell lines exhibited a 20% drop in productivity after passaging for approximately 4 weeks. These five NSO-derived production cell lines were successfully cultured to produce antibodies with acceptable product quality attributes in a standardized fed-batch bior- eactor process, consistently achieving an average specific productivity of 20-60 pg/cell-day, and a volumetric productivity exceeding 120. mg/L-day (Burky et al., 2006). In contrast to the commonly available NSO host cell line, which requires serum and cholesterol for growth; and the commonly used expression vector system, which uses a proprietary ***glutamine*** ***synthetase*** selection ****marker*** (GS-NSO), these NSO cells are cholesterol independent, grow

well in a protein-free medium, use a non-proprietary selection

marker , and do not require gene amplification for accept , and do not require gene amplification for productivity improvement: These characteristics are advantageous for use of this NSO cell line platform for manufacturing therapeutic antibodies.

L4 ANSWER 3 OF 9 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN AN 2007081036 EMBASE <<LOGINID::20070611>>

Derivation and characterization of cholesterol-independent non-GS NS0 cell lines for production of recombinant antibodies.

AU Hartman T.E.; Sar N.; Genereux K.; Barritt D.S.; He Y.; Burky J.E.; Wesson

M.C.; Tso J.Y.; Tsurushita N.; Zhou W.; Sauer P.W.

CS T.E. Hartman, Process Sciences and Engineering, PDL BioPharma, Inc.,

Campus Drive, Fremont, CA 94555, United States. taymar.hartman@pdl.com SO Biotechnology and Bioengineering, (1 Feb 2007) Vol. 96, No. 2, pp.

ISSN: 0006-3592 E-ISSN: 1097-0290 CODEN: BIBIAU

CY United States

DT Journal; Article
FS 022 Human Genetics
026 Immunology, Serology and Transplantation

LA English

English

ED Entered STN: 9 Mar 2007 Last Updated on STN: 9 Mar 2007

AB Presented is an antibody production platform based on the fed-batch culture of recombinant NS0-derived cell lines. NS0 host cells, obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK, Part No. 85110503), were first adapted to grow in a protein-free, cholesterol-free medium. The resulting host cell line was designated NSO-PFCF (protein-free, cholesterol-free). The five production cell lines presented here were generated using a common protocol consisting of transfection by electroporation and subcloning. The NSO-PFCF host cell line was transfected using a single expression vector containing the Escherichia coli xanthine-guanine phosphoribosyl transferase gene (gpt), and the antibody heavy and light chain genes driven by the ***CMV*** promoter. The five cell lines were chosen after one to three rounds of iterative subcloning, which resulted in a 19-64% increase in antibody productivity when four mother-daughter cell pairs were cultured in a fed-batch bioreactor process. The production cell lines were genetically characterized to determine antibody gene integrity, nucleotide sequences, copy number, and the number of insertion sites in the NS0 cell genome. Genetic characterization data indicate that each of the five production cell lines has a single stably integrated copy of the antibody expression vector, and that the antibody genes are correctly expressed. Stability of antibody production was evaluated for three of the five cell lines by comparing the early stage seed bank with the Working Cell Bank (WCB). Antibody productivity was shown to be stable in two of three cell lines evaluated, while one of the cell lines exhibited a 20% drop in productivity after passaging for approximately 4 weeks. These five NSO-derived production cell lines were successfully cultured to produce antibodies with acceptable product quality attributes in a standardized antibodies with acceptable product quality attributes in a standardized fed-batch bioreactor process, consistently achieving an average specific productivity of 20-60 pg/cell-day, and a volumetric productivity exceeding 120 mg/L-day (Burky et al., 2006). In contrast to the commonly available NSO host cell line, which requires serum and cholesterol for growth, and the commonly used expression vector system, which uses a proprietary ""glutamine"" ""synthetase*" selection ""marker"" (GS-NSO), these NSO cells are cholesterol-independent, grow well in a protein-free medium, use a non-proprietary selection ""marker", and on not require gene amplification for productivity improvement. These characteristics are advantageous for use of this NSO cell line platform for manufacturing therapeutic antibodies. .COPYRGT. 2006 Wiley Periodicals, Inc.

L4 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

AN 2007:95018 CAPLUS <<LOGINID::20070611>>

DN 146:336548

Derivation and characterization of cholesterol-independent non-GS NS0 cell lines for production of recombinant antibodies

AU Hartman, Taymar E.; Sar, Nalin; Genereux, Kimberly; Barritt, Diana S.; He, Yimin; Burky, John E.; Wesson, Mark C.; Tso, J. Yun; Tsurushita, Naoya; Zhou, Weichang; Sauer, Paul W.

CS Process Sciences and Engineering, PDL BioPharma, Inc., Fremont, CA, USA

SO Biotechnology and Bioengineering (2006), Volume Date 2007, 96(2), 294-306 CODEN: BIBIAU; ISSN: 0006-3592

PB John Wiley & Sons, Inc.

DT Journal

English

AB Presented is an antibody prodn. platform based on the fed-batch culture of recombinant NSO-derived cell lines. NSO host cells, obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK, Part No. 85110503), were first adapted to grow in a protein-free, cholesterol-free medium. The resulting host cell line was designated NS0-PFCF (protein-free, cholesterol-free). The five prodn. cell lines presented here were generated using a common protocol consisting of transfection by electroporation and subcloning. The NSO-PFCF host cell line was transfected using a single expression vector contg. the Escherichia coli xanthine-guanine phosphoribosyl transferase gene (gpt), and the antibody heavy and light chain genes driven by the ***CMV*** promoter. The five cell lines were chosen after one to three rounds of iterative subdoning, which resulted in a 19-64% increase in antibody productivity when four mother-daughter cell pairs were cultured in a fed-batch bioreactor process. The prodn. cell lines were genetically characterized to det. antibody gene integrity, nucleotide sequences, copy no., and the no. of insertion sites in the NS0 cell genome. Genetic characterization data indicate that each of the five prodn. cell lines has a single stably integrated copy of the antibody expression vector, and that the antibody genes are correctly expressed. Stability of antibody prodn. was evaluated for three of the five cell lines by comparing the early stage seed bank with the Working Cell Bank (WCB). Antibody productivity was shown to be stable in two of three cell lines evaluated, while one of the cell lines exhibited a 20% drop in productivity after passaging for approx. 4 wk These five NSO-derived prodn. cell lines were successfully cultured to produce antibodies with acceptable product quality attributes in a standardized fed-batch bioreactor process, consistently achieving an av. specific productivity of 20-60 pg/cell-day, and a volumetric productivity exceeding 120 mg/L-day. In contrast to the commonly available NS0 host cell line, which requires serum and cholesterol for growth, and the commonly used expression vector system, which uses a proprietary
glutamine ***synthetase*** selection ***marker*** (GS-NS0), these NS0 cells are cholesterol-independent, grow well in a protein-free medium, use a non-proprietary selection ***marker***, a do not require gene amplification for productivity improvement. These

characteristics are advantageous for use of this NS0 cell line platform for manufg, therapeutic antibodies.

RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN AN 2006:760190 CAPLUS <<LOGINID::20070611>> DN 145:329461

TI Method for rapidly constructing transgenic mammalian cell line with high-level expression of target gene
IN Huang, Ying; Wang, Yan; Shen, Beifen; Li, Yan
PA Beijing Tianguangshi Biotechnology Co., Ltd., Peop. Rep. China
SO Faming Zhuani Shenqing Gongkai Shuomingshu, 31pp.
CODEN: CNXXEV

DT Patent

LA Chinese FAN.CNT 1

PATENT NO. KIND DATE

APPLICATION NO. DATE

20051109 CN 2005-10064335 PRAI CN 2005-10064335

20050414
marker -integrated vector and a target AB The invention provides a gene expression vector for high-level expression of the target gene in mammalian cells. The ""marker"* vector contains a dominant selective ""marker"*, a screening ""marker"*, one or more amplification genes, genetic elements for proliferation, and recombination signal sequence (RSS). The expression vector contains a dominant selective ""marker", genetic elements, RSS which is identical with that in the ""marker" vector, a larget gene expression cassette, and replication origin of eukaryotic virus. The invention also provides a method for prepg, transgenic mammalian cell lines with stable and high-level expression of the target gene by steps of using the inventive ***marker*** vector to prep. cell lines in which active sites with high transcription level in the genome can be PCR amplified; co-transforming

the cell lines with the target gene expression vector and the recombinase expression vector, wherein the recombinase expression vector expresses RSS-specific recombinase, which catalyzes specific recombination at the above two RSS sites; and screening pos. transformants.

L4 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN AN 2004:80885 CAPLUS <<LOGINID::20070611>> DN 140:140659

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Vector containing viral cytomegalovirus promoter, mouse IgG2a gene, and 
***glutamine*** ***synthetase*** cDNA, and its use in transfecting 
CHO cells for recombinant protein production
 IN Kallmeier, Robert; Gay, Robert
PA Lonza Biologics Ptc., UK
               PCT Int. Appl., 49 pp.
          CODEN: PIXXD2
 DT Patent
 LA English
FAN.CNT 1
           PATENT NO.
                                                                      KIND DATE
                                                                                                                           APPLICATION NO.
 PI WO 2004009823
         WO 2004009823 A1 20040129 WO 2003-EP7946 20030721
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
CA 2489016 A1 20040129 CA 2003-2489016 20030721
RU 2003251434 A1 20040209 AU 2003-251434 20030721
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
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                         IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK

1668749 A 20050914 CN 2003-816914 20030

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 PRAI GB 2002-16648
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           WO 2003-FP7946
  AB The invention provides a CHO cell transformed with an expression vector
           comprising a: (a) promoter active in CHO cells and able to express a
          recombinant protein; (b) portion of the mouse IgG2a gene, which is able to enhance said promoter; and (c) selectable ""marker", such as ""glutamine" ""synthetase" gene. The invention provides the use of said transfected CHO cell in recombinant prodn. of a protein of
           interest. The invention relates that said promoter is a strong viral
         Interest. The invention relates that said promoter is a strong viral promoter, such as human cytomegalovirus (hCMV) promoter, and that said IgG2a gene lacks its naturally occurring promoter. The invention also relates that mouse ****CMV**** promoter can enhance transfection rate in CHO cells. As way of illustration, green fluorescent protein was expressed in CHO-K1 cells transfected with expression vectors contg. sequences for hCMV promoter and hCMV promoter in presence of IgG2a hot spot sequence. The invention provided the DNA sequences for some of the
              expression vectors used in this illustration.
CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS
 RE.CNT 6
                            ALL CITATIONS AVAILABLE IN THE RE FORMAT
  L4 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
 AN 2001:50811 CAPLUS <<LOGINID::20070611>> DN 134:111243
            Method for selecting high-expressing host cells using dicistronic
          expression system containing selectable/amplifiable gene within an intron Chisholm, Vanessa; Crowley, Craig W.; Krummen, Lynne A.; Meng, Yu-Ju G.
               Genentech, Inc., USA
 so
          PCT Int. Appl., 75 pp.
CODEN: PIXXD2
 DT Patent
  LA English
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                                                                                                                           APPLICATION NO.
          PATENT NO.
                                                                     KIND DATE
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                WO 2001004306 A1 20010118 WO 2000-US18841 20000711
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW
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                 TV., ZA, ZW

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CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

A 2385102 A1 20010118 CA 2000-2385102 20000711

P1196566 A1 20020417 EP 2000-945309 20000711
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JP 2003504059 T 20030204 JP 2001-509510
AT 317011 T 20060215 AT 2000-945309
ES 2257303 T3 20060801 ES 2000-945309
US 2005005310 A1 20050106 US 2003-714000
US 2007037254 A1 20070215 US 2006-535038
US 2007054303 A1 20070308 US 2006-535038
PRAI US 1999-143360P P 19990712
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              Vectors and methods for efficient isolation of recombinant cells
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expressing high levels of a desired protein are provided. The vectors comprise an amplifiable selectable gene, a fluorescent protein gene, and a gene encoding a desired product in a manner that optimizes transcriptional and translational linkage. The method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3 to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification continuing an amphining agent to solution to allow an implication to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified. CHO cells contg. Issue plasminogen activator (IPA) expression vectors according to the invention produced .gtoreq.9-fold higher IPA levels after amplification than did CHO cells contg. conventional vectors. The vector was a pRK deriv. This vector contains a cytomegalovirus immediate early promoter and an intron having a splice donor site derived from the cytomegalovirus immediate early gene and a splice acceptor site from an IgG heavy chain variable region gene. The DHFR gene was inserted into this intron and the IPA gene was inserted downstream of the splice acceptor site. THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS

RE.CNT 7 RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

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ANSWER 8 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN
AN 1999:779157 CAPLUS <<LOGINID::20070611>>
   132:19632
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Method for integrating genes at specific sites in mammalian cells via homologous recombination and vectors for accomplishing the same IN Reff, Mitchell R.; Barnett, Richard Spence; McLachlan, Karen Retta

Idec Pharmaceuticals Corporation, USA
U.S., 43 pp., Cont.-in-part of U.S. 5,830,698.
CODEN: USXXAM DT Patent LA English FAN.CNT 2 KIND DATE APPLICATION NO. PATENT NO. DATE 19991207 19981103 US 1998-23715 US 1997-819866 PI US 5998144 19980213 US 5830698 19970314 19980924 CA 1998-2283740 19980309 CA 2283740 20060627 WO 9841645 19980924 WO 1998-US3935 W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LY, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG AU 9864435 AU 737155 19981012 20010809 A B2 AU 1998-64435 19980309 EP 981637 20000301 EP 1998-910109 19980309 EP 981637 B1 20050525 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, BR 9808584 20000523 BR 1998-8584 19980309 A2 20001128 HU 2000-2320 T 20010925 JP 1998-540539 6 20040414 CZ 1999-3162 HU 200002320 19980309 JP 2001516221 CZ 293355 19980309 В6 19980309 20050615 AT 1998-910109 20050930 PT 1998-910109 AT 296356 19980309 PT 981637 19980309 RO 120148 20050930 RO 1999-972 19980309 20051116 ES 1998-910109 20051214 EP 2005-75757 19980309 19980309 ES 2242997

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI EP 1605055 20051214 EP 2005-76212 19980309 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI PL 191251 20060428 PL 1998-335695 19980309 IN 189732 20030419 IN 1998-DE616 19981009 ZA 1998-2152 19980310 ZA 9802152 19980313 TW 232239 20050511 TW 1998-87103778 19980313 20020702 US 6413777 B1 US 1999-343485 19990630 NO 9904397 19991109 NO 1999-4397 19990910 MX 9908363 US 2002192820 A 20000630 MX 1999-8363 A1 20021219 US 2002-109853 19990910 20020401 US 6841383 US 2004166528 B2 20050111 20040826 US 2004-817950 A1 20040406 IN 2004DE02321 20060922 IN 2004-DE2321 20041119 A2 19970314

PRAI US 1997-819866 US 1998-23715 EP 1998-910109 WO 1998-US3935 19980213 19980309 19980309 A3 W 19980310 IN 1998-DE616 US 1999-343485 A1 19990630

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US 2002-109853
                                                              A1 20020401
 AB A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein
         a desired DNA is integrated at a predetd, transcriptionally active site previously marked with a ***marker*** plasmid (Desmond). This unique
        previously marked with a "marker" plasmid (Desmond). This unique site may be bacterial DNA, a viral DNA or synthetic DNA. This Desmond ""marker" plasmid contains the Salmonella HisD gene, the Neomycin phosphotransferase exon 3, the murine dihydrofolate reductase, cytomegalovirus and SV40 enhancers, splice acceptor site, mouse beta
         globin major promoter, bovine growth hormone polyadenylation site, SV40
         early and late polyadenylation sites. The selectable ***marker*
proteins may include neomycin phosphotransferase, histidinol
         dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, HSV thymidine kinase, adenosine deaminase, ***glutamine***
***synthetase***, and hypoxanthine-guanine phosphoribosyl transferase.
         Marked CHO cells were produced and characterized. Other cells that may be marked include myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells. The method is particularly suitable
         for the prodn. of mammalian cell lines which secrete mammalian proteins at high levels, in particular Igs. Novel targeting vectors (Molly) and vector combinations for use in the subject cloning method are also
         provided. This Molly vector contains dihydrofolatereductase, N1+Neomycin phosphotransferase exon1, N2+Neomycin phosphotransferase exon 2, anti-
       ight chain leader+variable, human kappa const., anti-CD20 heavy chain leader+variable, human gamma 1 const., Salmonella histidinol dehydrogenase, ""CMV"" and SV40 enhancers, SV40 origin, splice donor/acceptor, ""CMV"" promoter/enhancer, HSV TK promoter and poloma enhancer, mouse beta globin major promoter, SV40 late polyadenylation, bovine growth hormone polyadenylation. Expression of an Anti-CD20 and Anti-human CD23 antibody and immunoadhesin in Desmond
         CHO cells was achieved
  RE.CNT 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS
 RECORD
                       ALL CITATIONS AVAILABLE IN THE RE FORMAT
L4 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN AN 1998:228730 CAPLUS <<LOGINID::20070611>>
 DN 129:63681
DN 129:63681

II High-level expression of HBsAg in CHO cells using "***glutamine***

TI High-level expression of HBsAg in CHO cells using "***glutamine***

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SO Bingdu Xuebao (1997), 13(2), 103-109

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 PB Bingdu Xuebao Bianjibu
             Journal
       Chinese

""Clutamine" ""synthetase" gene (GS) was introduced into CHO cells. The transfectants were selected by growth in a glutamine-free medium. Vector amplification was subsequently selected using specific inhibitor of GS, methionine sulfoximine (MSX). Combining with the ""CMV" promoter, HBV S gene were expressed in CHO cells. After two sounds of selection for vector amplification, the expression level of G4
         rounds of selection for vector amplification, the expression level of G4 cell line was >1:256 (RPHA), which was twice of that of the B43 cell line
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---Logging off of STN---

obtained using dhfr gene amplification.

Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS

SINCÉ FILE TOTAL ENTRY SESSION 36.32 37.37

FULL ESTIMATED COST

36.32 37.37

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE

TOTAL

ENTRY SESSION

CA SUBSCRIBER PRICE

-5.46 -5.46

STN INTERNATIONAL LOGOFF AT 16:44:37 ON 11 JUN 2007